

## Diurnal cortisol rhythm as a predictor of lung cancer survival

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### ABSTRACT

**Background:** Poorly coordinated diurnal cortisol and circadian rest-activity rhythms predict earlier mortality in metastatic breast and colorectal cancer, respectively. We examined the prognostic value of the diurnal cortisol rhythm in lung cancer.

**Methods:** Lung cancer patients ( $n = 62$ , 34 female) were within 5 years of diagnosis and had primarily non small-cell lung cancer, with disease stage ranging from early to advanced. Saliva collected over two days allowed calculation of the diurnal cortisol slope and the cortisol awakening response (CAR). Lymphocyte numbers and subsets were measured by flow cytometry. Survival data were obtained for 57 patients. Cox Proportional Hazards analyses were used to test the prognostic value of the diurnal cortisol rhythm on survival calculated both from study entry and from initial diagnosis.

**Results:** The diurnal cortisol slope predicted subsequent survival over three years. Early mortality occurred among patients with relatively “flat” rhythms indicating lack of normal diurnal variation (Cox Proportional Hazards  $p = .009$ ). Cortisol slope also predicted survival time from initial diagnosis ( $p = .012$ ). Flattened profiles were linked with male gender ( $t = 2.04$ ,  $df = 59$ ,  $p = .046$ ) and low total and cytotoxic T cell lymphocyte counts ( $r = -.39$  and  $-.30$ ,  $p = .004$  and  $.035$ , respectively). After adjustment for possible confounding factors, diurnal slope remained a significant, independent predictor of survival. **Conclusions:** Flattening of the diurnal cortisol rhythm predicts early lung cancer death. Data contribute to growing evidence that circadian disruption accelerates tumor progression.

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### 1. Introduction

Among patients with advancing cancer, dysregulation of circadian physiology is often conspicuous and extensive. Disrupted circadian cycles are evident in endocrine, immune, metabolic, and cellular systems (Mazzocchi et al., 2010; Hrushesky, 1985; Mormont et al., 2000). Dysregulation of circadian hypothalamic–pituitary–adrenal (HPA) rhythms has been linked specifically with advancing cancer and accelerated tumor growth rates (Mormont and Lévi, 1997; Touitou et al., 1995; Hrushesky et al., 1998; Sephton and Spiegel 2003; Eismann et al., 2010).

Human cortisol peaks 30–45 min after first awakening and drops to a nadir during sleep (Clow et al., 2010). From 30% to 70% of patients with advanced cancer display idiosyncratic rhythm abnormalities including unsynchronized peaks and troughs, consistently high or low levels and erratic circadian fluctuations. Viewed in aggregate, the diurnal cortisol profiles of advanced cancer patients often appear “flattened” (Mormont and Lévi, 1997; Sephton et al., 2000). The diurnal cortisol slope captures multiple types of rhythm deviation and is measurable in saliva (Turner-Cobb et al., 2000; Kraemer et al., 2006; Hellhammer et al., 2009). Elevated diurnal cortisol slope predicts early metastatic breast cancer mortality, independent of other prognostic factors (Sephton et al., 2000). Flattened slopes were associated with low natural killer cell counts and cytotoxicity, sleep disruption, and marital dissolution; none of which explained the cortisol-survival relationship (Sephton et al., 2000). Circadian rest/activity rhythms are also irregular in patients with advancing cancer (Mormont and Lévi,

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1997), and are measurable by wrist-worn actigraphy (Ancoli-Israel et al., 2003). Poor circadian coordination of rest/activity rhythm predicts early mortality over four years in metastatic colorectal cancer patients (Mormont et al., 2000), a finding recently replicated in large multi-site study (Innominato et al., 2009). Among cancer patients, circadian disruption may be a preexisting cause, a correlate of distress and physiological burden, a direct effect of cancer on the brain, and/or a mediator of psychosocial effects on tumor progression (Sephton and Spiegel 2003; Eismann et al., 2010; Chida et al., 2008). A few clinical prospective studies have provided a fascinating demonstration of prognostic value for circadian rhythms in human cancers. These require replication and testing in other cancer types.

As compared with the comorbidities of other cancers, lung cancer-related distress, depression, fatigue, and sleep disruption are extreme (Parker et al., 2008). All these symptoms have been linked with circadian disruption in lung cancer, suggesting that this disease may convey uniquely high circadian disruption-mediated risk (Hrushesky et al., 2009). Lung cancer patients demonstrate markedly poor integration of circadian neuroendocrine-immune function evidenced by high evening cortisol, suppressed melatonin, and changes in the circadian patterns of distribution of peripheral lymphocytes (Mazzoccoli et al., 2003, 2005). Non-small cell lung cancer generally has a better prognosis than small-cell disease; however, five-year survival rates for both are poor (49–75% for early stage, and < 1% for advanced disease).

We examined the prognostic value of the diurnal salivary cortisol rhythm in lung cancer. Secondary analyses explored associations with potential explanatory and/or confounding factors including traditional prognostic indicators, depression, fatigue, and sleep quality. Additional biological factors were chosen based on their relevance in cancer studies of the effects of the stress, sleep, and circadian rhythms (Cole and Sood, 2012; Antoni et al., 2006; Eismann et al., 2010; Mazzoccoli et al., 2003, 2005). These included serum cortisol, sympathetic activation measured by over-night urinary catecholamines, lymphocyte counts and lymphocyte subsets.

## 2. Methods

### 2.1. Subjects and procedure

Eligible patients were at least 18 years old and had been diagnosed with Stage I–IV non-small cell lung cancer or with limited to extensive small cell lung cancer during the last five years. Exclusions were made for history of psychiatric hospitalization, alcohol abuse or dependence within the past two months, use of prednisone or concurrent medical conditions likely to influence short-term (six month) survival. A total of 223 patients were referred or approached for this study, 145 (65%) were eligible and of those eligible, 62 (42.7%) patients were enrolled after informed consent. Physician's diagnosis of lung cancer was confirmed. Reasons reported for refusal included feeling too ill, being too busy, or lack of interest.

Participants were scheduled for a one hour interview with a research assistant during which they provided informed consent, demographic, and medical information including smoking history and performance status. A blood sample was drawn and they were given instructions and materials for home-based saliva collection as well as self-report questionnaires. Home-based questionnaires assessed depressive symptoms, fatigue, and sleep difficulties. Eight saliva samples were collected over two consecutive days for measurement of diurnal salivary cortisol profiles. Fifteen-hour over-night urine collection provided estimates of catecholamine hormone release. Blood samples were drawn for flow cytometry.

Participants were provided with \$100 compensation when home-based data collection materials were returned. Medical data, including cancer stage at diagnosis, was collected both from medical chart review and from the Kentucky Cancer Registry.

#### 2.1.1. Sample characteristics

Study participants ranged in age from 41 to 84 years with a mean age of 64 years ( $SD = 9.12$ ). Thirty-four (55%) of those enrolled were female and 58 (94%) had non-small cell lung cancer. Eighty-five percent of the sample was Caucasian and 55% had twelve years of education or less. Forty-six percent of the sample was married and 25% were employed. Participants were a mean age of 62 years at the time of lung cancer diagnoses ( $SD = 8.9$ ). The median time since lung cancer diagnosis was 21 months, with a range of 1 month to 5 years. Six participants (10.7%) scored  $\geq 19$  on the BDI, which is consistent with clinically significant depressive symptoms. Table 1 displays the frequency and percentage of participants according to disease status. The sample included similar proportions of early (stage 1,  $n = 29$ ) and more advanced (stage 2–4,  $n = 33$ ) disease. Table 2 presents descriptive information for psychological and physiological variables.

Approximately two years after enrollment closed, data tracking for the study was closed and the investigators contacted the Kentucky Cancer Registry and consulted medical records for survival data. Registry and record review yielded documentation of death for 23 patients. Among patients who had died, median time from study entry to death was 317 days (range: 34 days to 2.7 years). Median time from diagnosis to death was 2.7 years (range: 7 months to 6 years). Causes of death included malignant neoplasms of the bronchus or lung ( $n = 11$ , 47.8%), chronic obstructive pulmonary disease ( $n = 1$ , 4.3%), and acute myocardial infarction ( $n = 1$ , 4.3%). Information on cause of death was unavailable for nine of those who died (39.1%). Among the entire sample, median follow up time from study entry was 2.2 years (range 34 days to 3.4 years). Median follow up from the date of lung cancer diagnosis was 4 years (range 7 months to 8.9 years).

### 2.2. Physiological measures

#### 2.2.1. Diurnal salivary cortisol

At the initial interview subjects received a saliva collection kit and were given instructions for collection. Kits included 8 pre-labeled collection tubes, or “salivette” devices (Walter Sarstedt Inc., Newton, North Carolina) with instructions on how and when to collect saliva samples and a questionnaire covering factors likely to introduce variation in cortisol levels (e.g., stressors, exercise). Subjects were instructed to sample saliva on two consecutive days. Saliva was collected at waking, 45 min after waking (45+), 1600, and 2100 h on each day. This enabled measurement of the diurnal cortisol slope, diurnal mean levels, the cortisol response to awakening (CAR), diurnal mean, area under the curve (AUC), and waking and evening levels. Subjects stored cortisol kits in their refrigerator until they were returned. Samples were centrifuged, aliquoted, and

**Table 1**  
Numbers of participants with each cancer type and stage.

	Frequency	Percent (%)
Non-small cell lung cancer	58	94
Stage I	27	44
II	7	11
III	17	27
IV	7	11
Small cell lung cancer <sup>a</sup>	4	6

<sup>a</sup> Among the four patients with small-cell lung cancer, two had limited, and two had extensive stage cancer.

**Table 2**

Mean and standard deviation (SD) values for psychological and physiological variables.

	N	Mean	SD
Diurnal cortisol slope log( $\mu\text{g}/\text{dL}$ )/Hr	61	−0.114	0.062
CAR log( $\mu\text{g}/\text{dL}$ )/Hr	58	0.705	1.426
BDI	61	10.5	8.2
PSQI	62	13.8	7.7
Serum cortisol $\mu\text{g}/\text{dL}$	55	14.5	8.0
Urinary Epinephrine, ng/mL/Hr	57	1.80	1.53
Urinary Norepinephrine, ng/mL/Hr	62	62.60	45.00
White blood cell count $\times 10^3/\mu\text{L}$	54	6.748	2.389
Absolute lymphocyte count $\times 10^3/\mu\text{L}$	54	1.494	0.662
CTL count $\times 10^3/\mu\text{L}$	52	0.344	0.224
NK cell count $\times 10^3/\mu\text{L}$	52	0.233	0.179

stored at  $-80^\circ\text{C}$  until assay. Cortisol levels were assessed using an enzyme immunoassay developed for use in saliva (Salimetrics, Inc., State College, PA). Assay sensitivity was  $0.007 \mu\text{g}/\text{dL}$ . The inter-assay coefficient of variation (CV) was 9.55% using the low control and 4.75% using the high control. Intra-assay CV average was 3.75% using the low control and 3.06% using the high control.

The diurnal cortisol slope was calculated by regressing log-transformed cortisol on sample collection time with 45 + samples excluded: these were used to calculate the cortisol awakening response (CAR; Kraemer et al., 2006). Since the distribution of raw cortisol values is typically skewed and the normal diurnal profile may be approximated by an exponential curve, raw values were log transformed. The regression of six of the values (waking, 1600, and 2100 collections over two days) upon the hour of sample collection was calculated. The unstandardized beta (slope of the regression) was used as a measure of the diurnal salivary cortisol rhythm (Kraemer et al., 2006). Mean log-transformed cortisol values were calculated. In accordance with recommendations that meaningful CAR measurement should include both the first waking sample plus a measure of change (Kraemer et al., 2006; Clow et al., 2010), the CAR was calculated by regressing log-transformed values of the first two samples (waking and 45 + over two days) upon collection time and using the unstandardized beta (slope of the regression) as a measure of CAR. Recommendations are based on the notion that diurnal slope and CAR are driven by somewhat different regulatory mechanisms. Both calculations involved the waking cortisol value and thereby share some variance; but post-waking samples were used only in the CAR calculation, allowing greater statistical distinction between these two outcomes and reflecting their different biological underpinnings.

### 2.2.2. Serum cortisol

A blood sample totaling less than 30 ml was obtained during the interview. Aliquots of 1 ml serum were frozen at  $-80^\circ\text{C}$  until enzyme-linked immunosorbent assays (ELISA) were conducted to determine levels of serum cortisol (ALPCO Diagnostics, Salem, NH). The sensitivity for the assay of serum cortisol was  $0.3 \mu\text{g}/\text{dL}$ . The inter-assay CV for serum cortisol was 14.5%. The intra-assay CV was 11.2%.

### 2.2.3. Sympathetic activation

Fifteen-hour urinary epinephrine and norepinephrine levels were measured as an indicator of systemic sympathetic activation. Participants were given urine collection containers and asked to complete a 15-h overnight urine collection from 5:00 pm to 8:00 am the next morning. Samples were kept in coolers until return, at which time urine volume was recorded. Four aliquots were frozen at  $-80^\circ\text{C}$  until ELISA assays for epinephrine and norepinephrine were performed (ALPCO Diagnostics, Windham, NC). The sensitivity, inter-assay CV, and intra-assay CV for urinary epinephrine were 0.33 ng/mL, 15.5%, and 11.6%, respectively. The sensitivity, inter-assay CV, and intra-assay CV for urinary norepinephrine were 1.33 ng/mL, 10.3% and 8.5%, respectively. Concentrations are expressed per hour of collection.

### 2.2.4. Lymphocyte counts

Whole blood samples were subjected to flow cytometry for measurement of white blood cell count, total lymphocyte count, CD3/CD8 cytotoxic T lymphocyte (CTL) count, and CD3-/CD56 natural killer (NK) cell count.

## 2.3. Psychosocial measures

### 2.3.1. Beck depression inventory (BDI)

The BDI is a 21-item questionnaire that is routinely used to assess affective, cognitive, motivational, behavioral, and biological symptoms of depression and has acceptable psychometric properties (Beck et al., 1988). Internal consistency for the BDI ranges from .73 to .92 with a mean of .86 (Beck et al., 1988). The BDI demonstrates alpha coefficients of .86 and .81 for psychiatric and non-psychiatric populations, respectively and has a split-half reliability coefficient of .93 (Beck et al., 1988). The summary score was used in this study.

### 2.3.2. Fatigue symptom inventory (FSI)

The FSI is a 13-item self-report measure designed to assess the intensity and duration of fatigue and its interference with quality of life (Hann et al., 1998). This instrument has demonstrated construct and convergent validity as well as high internal consistency (Hann et al., 1998, 2000). The FSI is established as a valid and reliable measure of fatigue in cancer patients (Hann et al., 1998, 2000). The fatigue interference with quality of life score was used in analyses.

### 2.3.3. Pittsburgh sleep quality index (PSQI)

The PSQI is a measure of sleep quality and disturbances over a 1-month time period (Buysse et al., 1989). Psychometric evaluation supports the internal consistency, reliability and construct validity of the PSQI and it has proven utility in cancer patient populations (Beck et al., 2004; Carpenter and Andrykowski, 1998). The global PSQI score was used in analyses, a higher score indicates poorer sleep quality.

## 2.4. Statistical methods

Survival information was available for 57 patients who remained in Kentucky. We received sufficient salivary data to calculate the diurnal cortisol slope for all but one of these participants. Survival was calculated both from each participant's date of study entry and date of initial diagnosis.

The primary hypothesis was tested by analyzing the relationship between baseline diurnal cortisol slope and subsequent survival time. Separate regressions were conducted on survival time measured from the date of study entry and date of initial diagnosis, using the Cox Proportional Hazards Model in two-tailed tests. These analyses were repeated after excluding the minority of subjects with small cell lung cancer.

Secondary hypotheses were exploratory in nature, and were tested with conservative intent, to rule out the possibility that any relationship between the cortisol slope and survival time was explained by variance due to some other factor related to the disease or treatment process. These hypotheses evaluated the possible contribution of traditional prognostic indicators, including cancer stage, performance status, age at diagnosis, time since diagnosis, gender, socioeconomic status, smoking, prior chemotherapy and radiotherapy, and additional possible explanatory and/or con-

founding variables, including depressive symptoms, fatigue, self-reported sleep, serum cortisol, overnight urinary catecholamine levels, and lymphocyte counts and subsets. Bivariate Spearman rank correlations with one-tailed tests were used with the intent of most conservatively identifying factors associated with the cortisol slope that might explain the relationship between cortisol rhythm and survival. For factors significantly associated with the diurnal cortisol rhythm, the contributions of these variables to the cortisol rhythm-survival relationship were tested by entering each variable as a covariate with the cortisol slope in a separate Cox regression. As a check, we also entered simultaneously into one Cox regression the cortisol slope with all potential confounding variables significantly associated with diurnal cortisol slope.

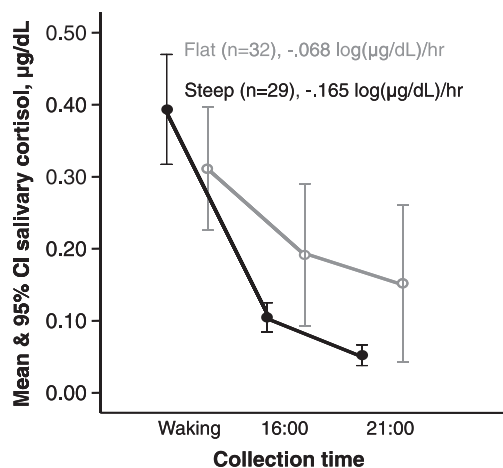
Because of tight connections between the hypothalamic central clock and the CAR response, we explored the predictive value of the CAR on survival with the rationale that it is also a possible indicator of circadian disruption. We tested the predictive value of cortisol AUC to assess possible effects of overall cortisol elevation as opposed to diurnal rhythmicity. The prognostic value of total lymphocyte and CTL counts was also tested because of previous data suggesting their potential value (Sephton et al., 2000; Blake-Mortimer et al., 2007).

Based on the effect sizes we reported previously (Sephton et al., 2000), a power estimate of 76% was predicted for this sample (Faul et al., 2007).

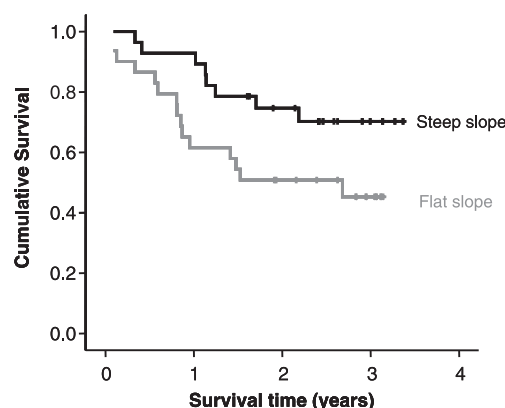
### 3. Results

The diurnal cortisol slope predicted subsequent survival over a period of up to 3 years. Early mortality occurred among patients with higher slopes, or relatively “flat” rhythms indicating lack of normal diurnal variation (Cox Proportional Hazards two-tailed  $p(\text{Wald}) = .009$ ; hazard ratio = 68052.8; 95% confidence interval (CI) = 15.481–299154617.4). Cortisol slope also predicted survival time from initial diagnosis ( $p = .019$ ; hazard ratio = 34867.4; 95% CI = 5.488–221531615.074). Results retained significance when primary analyses were repeated after removing the four subjects with small cell lung cancer ( $p = .050$ ). Neither the diurnal cortisol AUC nor the CAR were associated with subsequent survival time.

Although the survival analyses was conducted using the entire sample and cortisol slope as a continuous variable, for descriptive (not statistical) purposes, subjects were split into two equal groups based on a median split of the cortisol slope. Fig. 1 shows the mean raw cortisol levels at wake, 1600 and 2100 h for these two groups. Fig. 2 shows Kaplan–Meier survival curves of the two groups with



**Fig. 1.** For descriptive purposes, mean and 95% confidence interval raw diurnal salivary cortisol levels are shown across two days of saliva sample collection. Patients were split at the median diurnal cortisol slope,  $-.107 \log(\mu\text{g/dL})/\text{hr}$ .



**Fig. 2.** Kaplan–Meier survival curves for patients split at the median diurnal cortisol slope into two equal groups at the median diurnal cortisol slope. Cox proportional hazards models demonstrated that diurnal cortisol slope was prognostic for lung cancer survival over three years.

survival measured from the time of study entry. The divergence in survival as a function of slope emerged within the first year after cortisol assessment and extended at least three years after. Comparison of the two groups of patients split at the median cortisol slope reveals that 54% of those with flattened rhythms died, but death occurred in only 27% of those with comparatively rhythmic diurnal cortisol patterns. Survival plots of the two groups diverged significantly (log-rank,  $p = .046$ , two-tailed). Table 3 presents mean and SD numbers of lymphocytes and counts of lymphocyte subsets within groups split on the median diurnal cortisol slope.

Flattened cortisol rhythms were linked with more advanced lung cancer stage ( $r = .35$ ,  $p = .003$ ), poor performance status (Karnofsky;  $r = -.29$ ,  $p = .012$ ), male gender ( $t = 2.04$ ,  $df = 59$ ,  $p = .028$ ; Fig. 3) and low total lymphocytes and CTL  $r = -.39$  and  $-.30$ ,  $p = .002$  and  $.017$ , respectively). After adjustment for each of these factors, diurnal slope remained a significant, independent predictor of survival. As a check, we also entered simultaneously into a Cox regression the cortisol slope in addition to stage, Karnofsky rating, gender, total lymphocyte counts, and CTL counts. The diurnal cortisol slope retained significance in this analysis ( $p = .02$ ). When the sample was split by gender, the diurnal cortisol slope retained prognostic significance only among women ( $p = .014$ ), while it lost significance among the small group of male cancer patients.

Analyses revealed no significant associations of diurnal cortisol slope with the CAR or with age at diagnosis, cancer type, time since radio- or chemotherapy, socioeconomic status, prior marital disruption, depressive symptoms, fatigue, or sleep difficulties. The diurnal salivary cortisol slope was not associated with serum cortisol levels, urinary catecholamine levels, or NK cell counts.

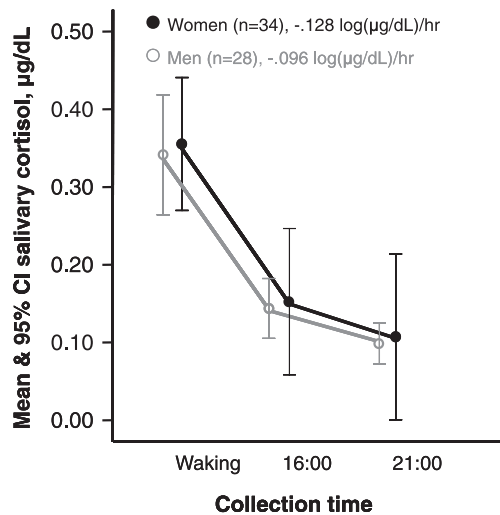
### 4. Discussion

Flattening of the diurnal cortisol rhythm predicted early mortality among lung cancer patients, a replication of our metastatic

**Table 3**

Mean and SD absolute lymphocyte counts and subsets within groups of lung cancer patients who were split at the median diurnal cortisol slope (cortisol and survival data for this grouping of participants are shown in Figs. 1 and 2).

	Diurnal cortisol slope			
	Steep		Flat	
	Mean	SD	Mean	SD
White blood cell count $\times 10^3/\mu\text{L}$	6.064	1.930	7.289	2.648
Total lymphocyte count $\times 10^3/\mu\text{L}$	1.604	0.632	1.361	0.661
CTL count $\times 10^3/\mu\text{L}$	0.416	0.239	0.246	0.179
NK cell count $\times 10^3/\mu\text{L}$	0.216	0.163	0.249	0.198



**Fig. 3.** For descriptive purposes, mean raw cortisol levels are shown at waking, 1600 and 2100 h for male and female patients. Cortisol rhythms examined by gender revealed that men had significantly higher (flatter) slope values than women,  $t(59) = 2.039$ ,  $p = .04$ .

breast cancer finding (Sephton et al., 2000), and recently noted in patients with metastatic renal cell carcinoma (Cohen et al., 2012). As originally observed in breast cancer patients, early mortality occurred in patients with relatively flat diurnal rhythms. These results are comparable with data from two clinical prospective studies demonstrating similar prognostic value for circadian rest/activity rhythms among colorectal cancer patients (Mormont et al., 2000; Innominato et al., 2009).

Cortisol and rest/activity rhythms are both strong markers of endogenous circadian phase with potential roles as zeitgebers: signals from the central clock that synchronize peripheral circadian rhythms (Reppert and Weaver, 2002; Mavroudis et al., 2012). The hypothalamic suprachiasmatic nucleus (SCN) coordinates rhythmic activities of peripheral cells using multiple signaling mechanisms including circadian rhythms of cortisol, behavior, and sympathetic nervous activity (Reppert and Weaver, 2002; Dickmeis and Foulkes, 2011; Lee et al., 2010; Gillette and Abbott, 2006; Balsalobre et al., 2000). Circadian signals regulate peripheral tumor suppressor and cell cycle genes that control cancer-relevant processes including cell proliferation, differentiation, and apoptosis (Lee et al., 2010). Recent data suggest the central circadian clock functions as a tumor suppressor (Fu and Lee, 2003).

#### 4.1. Circadian disruption can promote cancer incidence and accelerate tumor progression

New data from clinical, animal, cellular, and molecular studies reveal that central circadian disruption can promote cancer incidence and accelerate tumor growth (Sephton and Spiegel 2003; Eismann et al., 2010). Increased risk for both incidence and progression of human breast cancer is conferred by variants of circadian genes *Per3* and *NPAS2* (Zhu et al., 2005, 2008; Yi et al., 2010). Manipulating murine light–dark cycles can increase spontaneous and radiation-induced tumor development, speed implanted tumor growth, and cause early death (Lee et al., 2010; Filipowski et al., 2002). Mice with SCN lesions or central clock gene mutations suffer spontaneous tumor development and accelerated growth of implanted tumors (Fu and Lee, 2003; Filipowski et al., 2006, 2009). When the central clock is disturbed, HPA rhythms are altered and peripheral cellular clocks appear to lose control over cell proliferation, resulting in amplification of tumor growth rhythms (Lee et al., 2010; Yang et al., 2009a,b). Interestingly, tumor growth is doubled

under an experimental regimen that selectively disrupts circadian glucocorticoid rhythms (Filipowski et al., 2002).

Human data also point to circadian effects on tumor initiation and growth. Recent epidemiological results have been so convincing that the World Health Organization now concludes shift work is probably carcinogenic (2A) (Megdal et al., 2005; Straif et al., 2007).

#### 4.2. Cancer can initiate and exacerbate circadian disruption

Circadian–cancer relationships are bidirectional. Animal data show that cancer can cause circadian disruption. Tumors may become unresponsive to host circadian signals, disrupt the function of the central clock, and dysregulate peripheral circadian timing systems (Mormont and Lévi, 1997; Sephton and Spiegel, 2003; Eismann et al., 2010; Fu and Lee, 2003). Tumor burden-induced changes in physiological factors, including circulating cytokines, can disrupt circadian rhythms both in the brain and in the periphery. Further, human cancer and its treatments may profoundly disrupt circadian function (Mormont and Lévi, 1997; Sephton and Spiegel, 2003; Eismann et al., 2010): cancer-related anxiety and depression can induce sleep loss. Medication side effects, physical, existential, and interpersonal stressors can all exact a toll on circadian systems (Sephton and Spiegel 2003; Eismann et al., 2010; Chida and Steptoe, 2009). Given the bidirectional nature of effects, it is impossible to infer causality from the current prognostic data.

#### 4.3. Circadian disruption: an important factor in psycho-oncology

We also replicated correlations between flattened cortisol rhythms, more advanced cancer stage, and poor performance status (Mazzocchi et al., 2010; Sephton and Spiegel 2003; Eismann et al., 2010). Flattened diurnal cortisol rhythms have previously been noted among metastatic cancer patients with depression or emotional repression/anxiety (Jehn et al., 2006; Giese-Davis et al., 2004). Cancer patients with marked circadian disruption suffer greater fatigue, sleep disruption, anxiety, depression, and cognitive difficulties (Miller et al., 2008) as well as more intrusive thoughts and greater use of avoidant coping (Dedert et al., 2012). Concomitant alteration of cortisol secretion with elevated immune and inflammatory mediators has been observed in both cancer patients and cancer survivors (Sephton et al., 2009; Rich et al., 2005; Irwin et al., 2012; Bower et al., 2005a,b). Although these findings do not imply the direction of effects, such associations are important future considerations for psycho-oncology research (Porter, 2012).

Lung cancer prognosis is generally poorer in men than in women. The more marked cortisol disruption seen among males mirrors some, but not all data from healthy individuals (Saxbe et al., 2008; Larsson et al., 2009). Fig. 3 shows mean raw cortisol for male and female patients. Although men had significantly flatter diurnal profiles ( $t(59) = 2.039$ ,  $p = .04$ ), gender was not a significant predictor of survival, nor did it significantly alter the cortisol–survival relationship. The persistence of prognostic value for the cortisol rhythm among the subsample of female patients is remarkable, given the small number of subjects in this secondary analyses.

#### 4.4. Circadian effects in cancer-relevant psychoneuroimmune and endocrine pathways

This study was guided by our theoretical model of circadian effects in cancer-relevant psychoneuroimmune and endocrine pathways (Sephton and Spiegel 2003; Eismann et al., 2010).

As hypothesized, lung cancer patients with flattened cortisol rhythms had lower total lymphocyte and CTL counts. Similar associations have previously been noted among lung and metastatic

breast cancer patients (Mazzocchi et al., 2010; Sephton et al., 2000) as well as healthy subjects (Dimitrov et al., 2009). Low CTL counts have previously been shown to predict early cancer mortality (Blake-Mortimer et al., 2004), but this association was not observed here.

Links between hormonal and immune rhythm disturbances have been observed in numerous cancer types (Touitou et al., 1995; Touitou et al., 1996; Abercrombie et al., 2004; Mazzocchi et al., 2003, 2010; Mormont and Lévi, 1997).

Among cancer patients with flattened cortisol rhythms; suppressed functional cellular immunity and inflammation have been reported (Miller et al., 2008; Desantis et al., 2011; Mravec et al., 2008), and this group of symptoms has been linked with poor treatment outcome (Rich et al., 2005). Glucocorticoids may dysregulate immunity via circadian-immune communication (Arjona and Sarkar, 2008) or epigenetic mechanisms (Krukowski et al., 2011). In turn, cancer-related cytokine secretion may contribute to the dysregulation of circadian HPA rhythms (Raison et al., 2006).

A large prospective study recently demonstrated prognostic significance for flattened diurnal cortisol slope in cardiovascular death (Kumari et al., 2011), possibly reflecting associations of circadian disruption with inflammation. Similar to our results, this study found no predictive value for the CAR or diurnal cortisol AUC. The distinct prognostic value of the diurnal cortisol slope may be related to specific mechanisms of regulatory control. For example, the SCN and sympathetic nervous system are crucial regulators of both circadian and awakening glucocorticoid responses (Clow et al., 2010); but circadian cortisol is specifically responsive to pineal melatonin and the peripheral adrenal circadian clock, which controls diurnal sensitivity of the axis (Dickmeis, 2009; Son et al., 2008).

In contrast with previous studies among cancer samples (Weinrib et al., 2010; Miller et al., 2008); the diurnal cortisol slope was not associated with distress, fatigue, or sleep difficulties in this sample. Within lung cancer research, associations between circadian dysregulation and health-related quality of life have primarily been noted in advanced stage patients (Levin et al., 2005). The mixed-stage composition of our sample may not have lent itself to detection of this association.

#### 4.5. Limitations of this research

These data do not prove a causal connection between cortisol rhythm and lung cancer survival. The sample was relatively small, and this problem was compounded by the loss of some physiological data due to limited sample volumes and difficulty collecting blood from a few of the patients (Table 2).

#### 4.6. Future directions for research

Recent research has focused on clock genes as tumor suppressors (Reddy and O'Neill, 2010), but few studies have examined circadian impacts on tumor defense mechanisms or cellular factors that may support tumor growth (e.g., VEGF; Koyanagi et al., 2003).

Circadian effects in cancer-relevant psychoneuroendocrine and immune pathways should also be considered. We need to elucidate interrelationships of circadian, neuroendocrine and immune function and discover whether circadian-immune impairments can accelerate neoplastic disease (Logan and Sarkar, 2012; Scheff et al., 2010). Our model (Sephton and Spiegel, 2003; Eismann et al., 2010) and additional recent data (Cohen et al., 2012; Lyon et al., 2008) suggest comprehensive assessment of cytokines and other immune parameters will help describe these relationships, reveal mediating mechanisms, and inform treatment strategies. Recent data highlight multiple pathways by which stress may accelerate tumor progression, and include convincing

results regarding sympathetic neural pathways (Antoni et al., 2006; Cole and Sood, 2012). However, very little focus has been devoted to circadian disruption as a possible mediator of psychosocial effects on tumor progression (Chida et al., 2008; Sephton and Spiegel, 2003; Eismann et al., 2010). Cortisol rhythms are strong candidate mechanisms due to their prognostic value and potential to coordinate peripheral cell processes crucial to tumor growth (Sapolsky and Donnelly, 1985; Ben-Eliyahu et al., 1991). Poor coordination of cortisol rhythms has been associated with failure of negative feedback inhibition of the HPA axis, suggesting that central clock and downstream HPA dysregulation are interrelated in advanced cancer patients (Spiegel et al., 2006). Future studies should also consider the endogenous pulsatility of the HPA axis as an aspect of circadian regulation (Young et al., 2004).

Manipulating circadian biology may prove an important weapon in the anti-cancer arsenal (Gery and Koeffler, 2010). A number of clinical strategies are known to effectively synchronize the central clocks of normal individuals. It remains to be seen if these are effective in reversing cancer-related circadian disruption, and transmitting quality or quantity-of-life benefits (Hrushesky et al., 2009). Behavioral and/or pharmacologic interventions may productively be targeted at circadian phenomena including HPA rhythms, sleep, light exposure and melatonin, physical activity, dietary and social behavior (Porter, 2012; Eismann et al., 2010). A promising current application of circadian biology is chronomodulation of chemotherapy, in which the timing of drug delivery is modulated according to circadian rhythms of the cancer patient to increase efficacy and reduce toxicity (Hrushesky, 1985; Innominato et al., 2010). A recent meta-analysis of chronotherapy studies reported a significant survival benefit (Liao et al., 2010).

Lung cancer poses a range of challenges exemplified by elevated rates of anxiety and depression related to breathlessness and pain (Pasquini et al., 2006). This complex range of factors for lung cancer patients. Suggests a need for multifaceted interventions.

## 5. Conclusions

Animal, cell, and molecular data support bidirectional effects: tumors can cause circadian disruption, but circadian disruption can also accelerate tumor growth and mortality (Fu and Lee, 2003). Taken together; the prospective nature of the current data, the independence of the primary finding from influence of any other prognostic indicator, the consistency with prior work in other cancers, and the support of a growing body of evidence from animal and cellular studies allow some confidence in the conclusion that circadian dysregulation is associated with more rapid human cancer progression. Together with research on tumor-suppressive effects of the central clock and clinical studies showing benefit from chronomodulation in cancer treatment; these data point to disrupted HPA rhythms as a risk factor for cancer progression and an area for additional therapeutic opportunity.

## Conflict of Interest

The authors of this manuscript have nothing to declare.

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